June 1977

U S D A FOREST SERVICE
RESEARCH NOTE RM-343

FOREST SERVICE
U.S. DEPARTMENT OF AGRICULTURE

ROCKY MOUNTAIN FOREST AND RANGE EXPERIMENT STATION

The Effects of Endomycorrhizae on Growth of Rabbitbrush, Fourwing Saltbush, and Corn in Coal Mine Spoil Material[1]

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The influence of vesicular arbuscular endomycorrhizae formed by *Glomus fasciculatus* on the survival and growth of rubber rabbitbrush, fourwing saltbush, and corn on coal mine spoil material was studied. *G. fasciculatus* endomycorrhizae increased the survival and growth of rabbitbrush but had no effect on fourwing saltbush or corn.

Keywords: Endomycorrhizae, *Chrysothamnus nauseosus, Atriplex canescens,* mine spoil reclamation.

Introduction

There has been only limited research on the mycorrhizal associations of plants colonizing strip-mined land and mining waste materials; however, the ecological importance of these associations has been suggested by several researchers (Aldon and Springfield 1975, Daft and Nicolson 1974, Daft, Hacskaylo and Nicolson 1975, Marx 1975, Schramm 1966). Schramm (1966) concluded that successful colonists on waste material were either nitrogen-fixing plants or ectomycorrhizal trees; however, endotrophic mycorrhiza were present, though rare, on these wastes. Marx (1975) has established that *Pisolithus* spp. ectomycorrhizae are instrumental in establishment and maintenance of pines on strip-mined spoils. Daft et al. (1975) found that grasses and other herbaceous plants colonizing the anthracite and bituminous coal spoils in Pennsylvania and bituminous coal spoils in Scotland generally were infected with endomycorrhizal fungi. These workers demonstrated that an endomycorrhizal fungus, *Gigaspora gigantea* (Nicol. and Gerd.) Gerdemann and Trappe, comb. nov., from Pennsylvania coal spoil stimulated the growth of corn in sand culture. They also found that *Glomus macrocarpus* var. *geosporus* (Nicol. and Gerd.) Gerdemann and Trappe, comb. nov., from coal spoil in Scotland stimulated the growth of

[1]*The research reported here is a contribution to the SEAM program. SEAM, an acronym for Surface Environment and Mining, is a USDA Forest Service program to research, develop, and apply technology that will help maintain a quality environment and other surface values while helping meet the Nation's mineral requirements. Journal Series 613, Agricultural Experiment Station, New Mexico State University, Las Cruces. This research was supported in part by USDA Forest Service, Grant No. 16-415-CA.*

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wild strawberry growing in pots containing sterilized spoil material. Aldon (1975) found that endomycorrhizae increased survival and growth of fourwing saltbush on strip-mined coal spoils in New Mexico.

The investigations described herein were undertaken to determine the effect of endomycorrhizae on the survival and growth of plants on coal spoil material collected from the McKinley coal strip-mine in northwestern New Mexico.

Materials and Methods

Growth Medium. Spoil material was collected in July 1975 from a recently graded spoil bank on the McKinley coal strip-mine near Gallup, New Mexico. Spoil material to the depth of 15 cm was collected and thoroughly mixed. Approximately half of the spoil material was autoclaved for 8 hrs at 121° C, and then spread out on a greenhouse bench to aerate for 7 days. The nonsterilized and sterilized spoil material were stored in 76-l metal containers in the greenhouse. The spoil material possessed the following characteristics: pH (saturated paste), 6.7; electrical conductivity, 4.5 x 10^3 mmhos; Ca, 32.6 meq/l; Mg, 18.8 meq/l; Na, 19.1 meq/l; sodium adsorption ratio, 3.8; extractable K, 0.33 meq/100g; extractable NO_3, 320 ppm; extractable P ($NaHCO_3$), 0.52 ppm; and organic matter, 6.7%.

Experimental Plants. Three plant species were selected: corn, *Zea mays* L. (Funk's 4384A), rubber rabbitbrush, *Chrysothamnus nauseosus* (Pall.) Britton, and fourwing saltbush, *Atriplex canescens* (Pursh.) Nutt. The latter two species are shrubs native to the area surrounding the McKinley mine. Seeds of these plants were placed on the surface of spoil material in ten 20-cm plastic pots containing 1,500 g spoil material and covered with a layer of sterile river sand. After 3 weeks, plants were thinned to one plant/pot. Plants were grown under greenhouse conditions and watered with tap water.

Endomycorrhizal Fungi. The mycorrhizal inoculum was obtained from soil collected around rabbitbrush and fourwing saltbush plants growing on nondisturbed sites near the spoil banks. Chlamydospores of *Glomus fasciculatus* (Thaxter sensu Gerdemann) Gerdemann and Trappe, comb. nov., plus a small number of *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe, comb. nov., were extracted from 250 cc soil by the wet sieving and decanting method of Gerdemann and Nicolson (1963). Infestation of spoil material with mycorrhizal fungi was accomplished by thoroughly mixing the spoil material in each pot with chlamydospores extracted from 250 cc soil.

To evaluate the effect of the mycoflora other than mycorrhizal fungi on plant growth in spoil material, soil washings (in each case 75 ml of the above soil suspension which passed through a 45μ mesh screen) were added to pots with spoil material.

Growth Measurements. Plant shoots were cut off at the soil line at the termination of the study and heights were measured (base of stem to apical meristem). Shoots were dried at 80° C for 48 hrs and then weighed.

Effects of Phosphorus Fertilization. To determine the effect of phosphorus alone on the growth of the three host plants in nonsterilized spoil material, the plants were grown in pots containing the following amounts of 0-46-0 phosphorus fertilizer: 0 g/pot, 0.3 g/pot and 1.0 g/pot. Rabbitbrush (three replications) was grown for 137 days, fourwing saltbush (five replications) for 137 days, and corn plants (two replications) for 110 days.

Chemical Analysis. The dried plant material from each pot was ground in a Wiley tissue mill with a #40 screen. Tissue samples were digested as reported by Throneberry (1974) and phosphorus content determined spectrophotometrically using a modification of the Murphy and Riley method (1962). Nitrogen content was determined by Winkler's Kjeldahl method as modified by Ma and Zuazaga (1942). Zinc and manganese contents were determined by atomic absorption spectrophotometer.

Determination of Mycorrhizal Condition. The presence of a mycorrhizal association was determined by microscopic examination of stained roots. Roots were cleared and stained with cotton blue (Bevege 1968) and subsequently examined for the presence of coenocytic hyphae, arbuscules and vesicles.

Results

Rabbitbrush. Rabbitbrush plants were grown for 132 days in sterilized and in nonsterilized mine spoil materials receiving the following treatments: control, soil washings and mycorrhizal inoculum. After 132 days, plant growth on sterilized and nonsterilized spoil material was similar (table 1). Endomycorrhizae were established only

Table 1.—Effect of endomycorrhizae on rabbitbrush grown in mine spoil material for 132 days.

Treatment	Mycorrhizal condition	Height	Dry weight	N	P	MN	ZN
		cm	*µg/g*	..... %		.... *µg/g*	
Sterilized spoil material							
Control	—	1.2a[1]	11a	—[2]	—	—	—
Soil washings	—	1.9a	20a	—	—	—	—
Mycorrhizal inoculum	+	6.7b	149b	1.78	0.148	58	327
Nonsterilized spoil material							
Control	—	1.5a	36a	—	—	—	—
Soil washings	—	1.5a	22a	—	—	—	—
Mycorrhizal inoculum	+	5.5b	127b	1.16	0.179	54	180

[1]*Means in the same column followed by the same letter do not differ significantly at 1% level.*
[2]*Insufficient tissue for chemical analysis.*

in those plants growing in spoil material receiving endomycorrhizal fungus inoculum. Coencytic hyphae and vesicles were observed in roots.

Mycorrhizal plants growing in both the sterilized and nonsterilized material had significantly greater dry weights and heights than those plants without endomycorrhizal fungi. Insufficient tissue was obtained from plants in the control and soil washing treatments for N, P, Mn and Zn analysis; therefore, comparisons could not be made between mycorrhizal and nonmycorrhizal plants for these elements. A repeat experiment showed similar results.

Figure 2.—Rabbitbrush seedlings grown in uninoculated, sterile mine spoil material (left) and sterile mycorrhizal spoil (right).

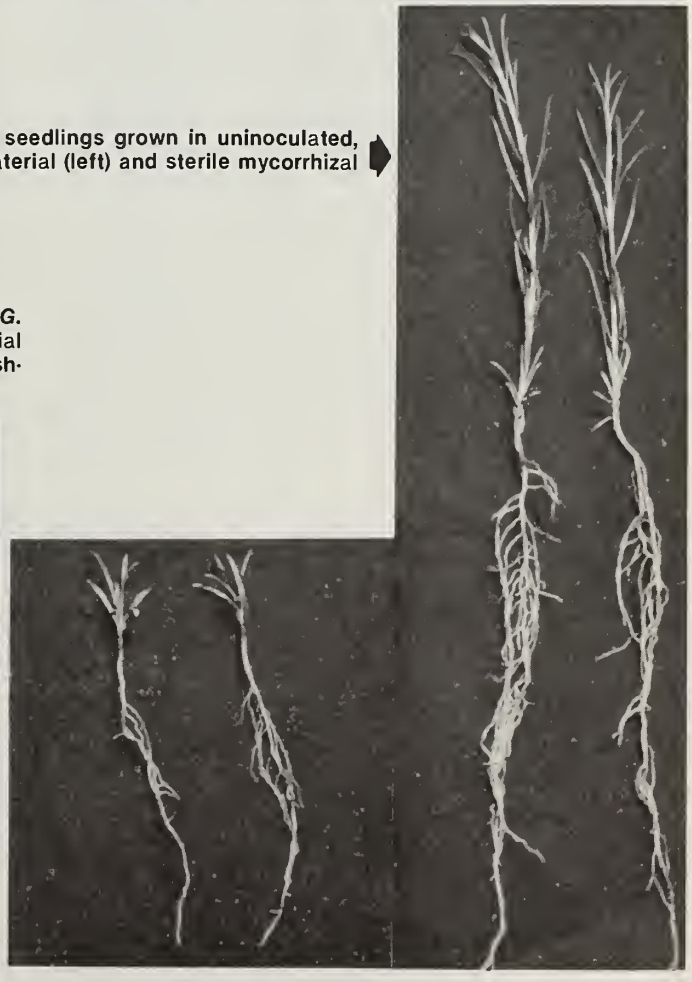
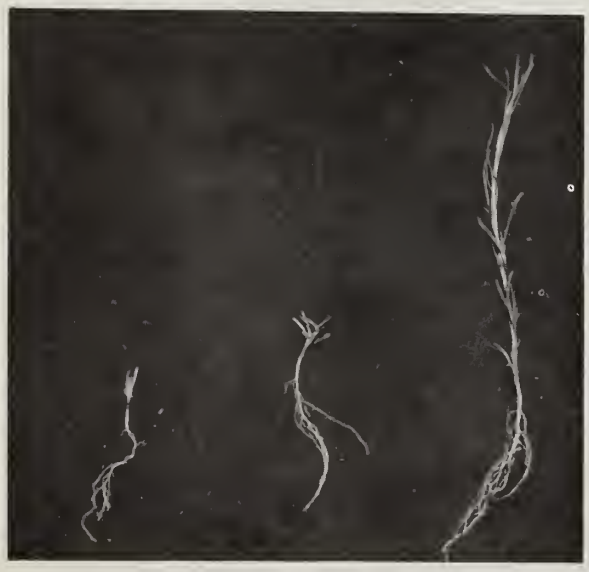


Figure 1.—Effect of inoculating mine spoil material with *G. fasciculatus* on rabbitbrush seedlings. Spoil material from left to right: nonsterile control, nonsterile washings, and nonsterile mycorrhizal.



The mycorrhizal growth response was first observed 10 weeks after plants had emerged. In the first and second experiments, approximately 25% and 60%, respectively, of the nonmycorrhizal plants died after becoming established. In contrast, no mycorrhizal plants died in the first experiment and only one died in the second experiment, after establishment.

G. fasciculatus spores were the predominant ones found in the spoil material surrounding the roots of the mycorrhizal plants at the end of the experiment. The only other type of endomycorrhizal fungal spore observed in this material, and only occasionally, belonged to *G. mosseae.*

Fourwing Saltbush. After 119 days, endomycorrhizal association were not established in any of the plants in this experiment, even in the treatments receiving mycorrhizal inoculum. Microscopic examination of cleared and stained roots revealed no typical mycorrhizal structure or stained coenocytic hyphae. These plants grew very little and the lower leaves had fallen off at harvest. No differences in height or dry weight were noted between treatments. Plant survival was 100% in all treatments. Similar results were obtained when this experiment was repeated.

Corn. After 94 days, little difference was noted in plant growth in the sterilized and nonsterilized spoil material (table 2). Only plants growing in spoil material receiving mycorrhizal inoculum were found to have endomycorrhizae.

There were no differences in height, dry weight or nitrogen and phosphorus content between mycorrhizal plants and plants grown in spoil material receiving soil washings. The mycorrhizal plants were significantly taller and had a greater dry weight than the control plants. No difference in phosphorus content of the control and mycorrhizal plants was found. There was no difference in the nitrogen content of the control and mycorrhizal plants in the sterilized spoil material; however, the control plants in the nonsterilized spoil material contained significantly more nitrogen than the mycorrhizal plants. In a repeat of this experiment similar results were obtained.

Corn plant survival was 100% in all treatments but leaves exhibited typical phosphorus deficiency symptoms 3 weeks after emerging. Senescence of lower leaves occurred after 6 weeks in all treatments, and at the end of the experiment only the terminal three leaves were functioning.

Phosphorus Test. Rabbitbrush plants growing in mine spoil material receiving phosphorus fertilizer were approximately 10 times taller and had 35 times greater dry weight than the control plants (table 3). There was little difference in the height and dry weight of the rabbitbrush plants receiving the two rates of P fertilizer.

Little difference in fourwing saltbush plant height was observed between the plants receiving

Table 2.—Effect of endomycorrhizae on corn grown in mine spoil material for 94 days.

Treatment	Mycorrhizal condition	Height	Dry weight	N	P
		cm	*g*	..... %	
Sterilized spoil material					
Control	—	15.7a[1]	1.9ab[2]	0.90a[2]	0.034a[2]
Soil washings	—	23.0b	2.4bc	0.58b	0.028a
Mycorrhizal inoculum	+	23.0b	2.7c	0.81ab	0.034a
Nonsterilized spoil material					
Control	—	15.5a	1.4a	1.14a	0.040a
Soil washings	—	24.8b	2.4bc	0.69b	0.023a
Mycorrhizal inoculum	+	26.3b	2.2bc	0.67b	0.048a

[1]*Means in the same column followed by the same letter do not differ significantly at 1% level.*
[2]*Means followed by the same letter do not differ significantly at 5% level.*

no P fertilizer and those receiving P fertilizer (table 3). Plants receiving P fertilizer had a greater dry weight than the control plants. Plants receiving the high fertilizer rate were approximately twice the weight of the control plants.

Corn plants grown in the spoil material with the low phosphorus rate were about 4 times taller and had 5 times greater dry weight than the control plants (table 3). Those plants receiving the high phosphorus rate were about 6 times taller and had 7 times greater dry weight than the control plants.

Discussion

Evidence presented in this paper further supports the theory of Aldon (1975), Daft and Nicolson (1974), and Daft et al. (1975) of the ecological importance of endomycorrhizal associations to plants initially colonizing mine spoil wastes in the West. The mycorrhizal fungus, *G. fasciculatus,* was found here to significantly increase survival and growth of rabbitbrush in sterilized and nonsterilized spoil material characterized by a very low phosphorus content (0.52 ppm extractable P) but with a pH (6.7) and nitrogen content (320 ppm NO_3) adequate for normal plant growth. However, this mycorrhizal fungus did not stimulate the growth of corn and fourwing saltbush in the same spoil material.

The survival and the growth response of rabbitbrush to mycorrhizal infection was attributed to an increase in uptake of phosphorus. This claim is indirectly substantiated by the significant increase in growth of rabbitbrush, corn and fourwing saltbush when phosphorus fertilizer was added to the spoil material.

The failure of *G. fasciculatus* to enhance the growth of fourwing saltbush apparently resulted from the inability of the fungus to infect the roots in the spoil material. This appears to be a type of host-microorganism specificity reaction (Mosse 1975), since Aldon (1975) found that *G. mosseae* was able to infect and increase the survival and growth of fourwing saltbush on McKinley mine spoil banks.

Figure 3.—Corn plants grown in mine spoil material showing effects of 1.0, 0.3, and 0 g/pot of a 0-46-0 phosphorus fertilizer.

Table 3.—Effect of three levels of phosphorus fertilizer (0-46-0) on height (*cm*) and dry weight (*mg*) of rabbitbrush, fourwing saltbush, and corn.

Fertilizer level (*g/pot*)	Rabbitbrush[1]		Fourwing saltbush[1]		Corn[2]	
	Height	Weight	Height	Weight	Height	Weight
0	2.3a[3]	8a	10.3a	225a	14.0a	1200a
0.3	28.0b	276b	12.5a	345b	52.5b	6200b
1.0	28.0b	319b	12.5a	487c	76.3c	8500c

[1]*Grown for 137 days.*
[2]*Grown for 110 days.*
[3]*Column means followed by the same letter do not differ significantly at the 1% level.*

Another type of mycorrhizal specificity, effectivity (Mosse 1975) was evident with corn. *G. fasciculatus* was able to infect corn roots as evidenced by hyphae, arbuscules and vesicles in the roots, but did not increase phosphorus uptake or growth. In this experiment, the mycorrhizal plants were taller and had a greater dry weight than the nonmycorrhizal plants. However, this response could not be attributed to a mycorrhizal association since nonmycorrhizal plants growing in spoil material receiving soil washing also exhibited the same growth response.

This study suggests that rabbitbrush should be mycorrhizal prior to field planting. It also indicates that more detailed work is needed on specificity in endomycorrhizae as it influences the establishment of plants on strip-mined wastes.

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